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Broadening the approaches to developing more effective vaccines

Lorne A. Babiuk *

Veterinary Infectious Disease Organization, 120 Veterinary Road, Saskatoon, Sask., Canada S7N 5E3

Abstract

Although vaccination has had a dramatic impact on reducing economic losses due to infectious diseases, vaccination technology has not changed dramatically over the last 200 years. However, with the advent of biotechnology and our understanding of virulence factors of infectious agents combined with our knowledge of the host immune response, we are now witnessing a revolution in the number of new agents which may potentially be controlled by vaccination, as well as the approaches being used to develop vaccines. These approaches include subunit vaccines, genetically modified live vaccines and most recently, polynucleotide vaccines. Pathogens involved in bovine respiratory disease are used as models to describe recent advances in developing new vaccines that have the potential to be safer, more economical and more efficacious. Emphasis will be placed on identification of specific proteins involved in inducing protective immunity and producing these in a mammalian expression system as subunit vaccines formulated with adjuvants. To increase the duration of immunity, the genes encoding these antigens have been introduced directly into animals as polynucleotide vaccines. The benefits and short-comings, as well as the practical problems associated (both scientific and regulatory) with eventual acceptance of these vaccines, are discussed.

Keywords: Vaccines; Genetic engineering; Marker vaccines

1. Introduction

Immunization has, over the last 200 years, contributed more to increasing the lifespan of humans than all the other impressive medical advancements. Furthermore, it is estimated that immunization has had a greater impact on the economics of livestock and poultry production than all other therapeutic and prophylactic treatments combined. Thus, even with the significant advancements in modern medicine, vaccination continues to be the most cost-effective method to reduce economic loss and animal suffering from infectious diseases. These spectacular statistics have been achieved by using either killed or attenuated conventional vaccines. Indeed, this success was possible even without fully comprehending the epidemiology and pathogenic mechanisms involved in the disease process. However, with the recent advances in our understanding of pathogenesis, primarily due to advances in immunology and molecular biology, we are in an era of unprecedented opportunity to develop safer and more effective vaccines to help further reduce diseases caused by infectious agents in humans and animals. The present review will focus on the different types of genetically engineered vaccines that are presently at different stages of development, clinical trials, or licensing. These include: (1) live vaccines, (2) live chimeric vaccines, (3) live replication defective vaccines, (4) subunit vaccines either developed as monovalent, multivalent or chimeric subunits, (5) peptide vaccines and the most recent addition to the armamentarium of vaccinologists (6) polynucleotide vaccines.

2. Some parameters of an ideal vaccine

Although the ideal vaccine for humans or animals may have some subtle differences, most of the desired parameters are identical regardless of species. These are summarized in Table 1. The primary concern in vaccination is efficacy and safety. Thus, vaccination itself must not cause any adverse reactions and should result in greater than 90% efficacy after a single

* Tel.: +1-306-966-7475; fax: 1-306-966-7478; e-mail: babiuk@sask.usask.ca.

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Table 1
Parameters of an ideal vaccine

(1) Efficacy greater than 90%
(2) Effective after a single dose
(3) Long-lived immunity (hopefully life-long)
(4) Effective when given orally (no need for injections)
(5) Induces a wide range of appropriate responses (mucosal, humoral, cellular)
(6) Low cost
(7) Compatible with local management practices
(8) Compatible with co-administration of other vaccines
(9) Stable (genetically/thermally)
(10) High safety

administration regardless of species or type of vaccine. Protection should occur rapidly, preferably within 2 weeks of administration and should be of long duration so that multiple boosters are not required. This reduces both the cost of immunization and improves overall compliance. By inducing immunity of long duration, it should further reduce the quantities of pathogen circulation due to 'herd immunity'. In order to increase compliance, development of multiple component vaccines are desirable since they will improve vaccine coverage as well as reduce the cost of immunization. Unfortunately, this is not easy to achieve due to potential interference between some types of vaccines and the need for better delivery systems. Significant progress is, however, being made in developing better vaccine delivery technologies which should improve the efficacy of multicomponent vaccines as well as simultaneously inducing both humoral and cellular immunity.

In many cases, it is unsatisfactory to introduce vaccines by intramuscular or subcutaneous routes. This is both expensive, traumatic for some individuals and may lead to injection site reactions. In commercial animal environments, where restraining a large number of animals is required, intramuscular injection often leads to significant tissue damage including broken needles which may be deposited in the muscle of the animals. Thus, it is imperative that better vaccine delivery systems are developed which reduce these unwanted side-effects. Oral or intranasal delivery is an attractive approach to vaccine delivery for all species. A further advantage of oral or intranasal delivery is that most infectious agents enter via mucosal surfaces, thus, immunity at these sites would prevent initiation of infection. In addition to developing mucosal immunity, the vaccine should develop a balanced immune response including both humoral and cellular immunity. In all instances, vaccines need to be genetically stable with no chance of reversion to virulence and induction of clinical signs in vaccinated individuals or in unvaccinated contacts. Finally, if vaccines could be developed which do not require a 'cold chain', this would dramatically improve vaccine efficacy.

3. Subunit vaccines

Subunit vaccines are defined as those containing one or more pure or semi-pure antigens. In order to develop subunit vaccines it is critical to identify the individual components, out of a myriad of proteins and glycoproteins of the pathogen, that are involved in inducing protection. Indeed, some proteins, if included in a vaccine, may be immunosuppressive, whereas in other cases immune responses to some proteins may actually enhance disease. Thus, it is critical to identify those proteins that are important for inducing protection and eliminate the others. In addition, it is critical to identify which immune responses are involved in preventing initiation of infection or clearing the disease. Since immunization generally does not induce sterile immunity, it is important to induce immunity to both reduce infection as well as help clear the infection rapidly and prevent disease if infection occurs. These are all issues that need to be addressed in designing any vaccination strategy.

The potential advantage of using subunits as vaccines are the increased safety, less antigenic competition, since only a few components are included in the vaccine, ability to target the vaccine to the site where immunity is required and the ability to differentiate vaccinated from infected animals (marker vaccines, see Section 4). Although subunit vaccines can be produced by conventional technologies, the economics of purification are generally not cost-effective due to the low quantities of protective antigens produced by the infectious agent. For example, *Pasteurella haemolytica* produces an extracellular toxin (leukotoxin) which is involved in pathogenesis and tissue damage in the lungs of cattle [1,2]. Although it is possible to isolate and purify the leukotoxin from conventional cultures [3], it is much more economical to use recombinant DNA technology to produce large quantities of the leukotoxin for incorporation into vaccines [4]. In addition, in vitro cultivation of the pathogen (especially bacteria) does not always result in the production of the protective antigen. For example, some bacterial proteins required for scavenging nutrients such as iron are not expressed at sufficient levels to allow purification of the protective component [5,6]. By identifying the proteins involved in inducing protective immunity and isolating the gene coding for these proteins it is possible to use recombinant DNA technology or synthetic peptide technology to produce sufficient quantities of these protective epitopes for incorporation into vaccines.

In addition to identifying potential targets of pathogens, it is also possible to develop subunit vaccines which reduce vector transmission of some pathogens by immunization against the vector. For example, many infectious agents are transmitted by blood feeding vectors. Studies have shown that immunization of animals against antigens present in the gut of the vector results in the disruption of intestinal cell function of the vector, after exposure to blood containing antibodies directed against these intestinal antigens [7]. This leads to death of the vector or a dramatic reduction of their reproductive capacity. For example, an 86K glycoprotein was identified in the mid-gut of ticks and has been used successfully as a vaccine to reduce tick infestation. Under normal circumstances, the host is never exposed to these 'concealed' antigens, therefore, immunity will not develop against these antigens even after repeated infestation. Thus, it is critical to produce these antigens in vitro and introduce them as vaccines into the animal. These approaches are directed at reducing the rate of spread of disease rather than against the disease itself.

It is fortunate that progress in the last few years has identified proteins or glycoproteins that are important in inducing protective immunity from many families of pathogens. Armed with this information, it is relatively easy to draw inferences regarding potentially important proteins of other members of the same families (functional genomics and proteomics) and to clone these genes. Thus, one no longer needs to investigate all the proteins/glycoproteins or the physiology of the pathogens to determine which ones might be critical for incorporation in a vaccine. This information is shortening the time required to develop subunit vaccines for a large number of pathogens.

Once the putative protective proteins are identified, they must be expressed in various expression systems. In the case of bacterial proteins, the most efficient expression systems are prokaryotic systems. Indeed, in some cases up to 40% of the newly synthesized protein in the bacteria are the protein of choice (vaccine). Either gram-negative or gram-positive organisms can be engineered to secrete the proteins into the growth media, target the product into the paraplasm, or have the bacteria retain the product intracellularly. If the product is retained, the protein is often present as an insoluble protein mass (inclusion bodies), which requires solubilization and refolding into the native state. Production of protein as inclusion bodies has some advantages in that the purification steps are relatively easy. However, the downstream processing requiring solubilization and refolding may be major impediments for some proteins. In contrast, if the product is secreted it generally does not require refolding but may require additional purification to remove undesired protein contaminants from the culture fluids. The exact choice of expression system used will be dependent on the characteristics of the protein being expressed. Since these foreign proteins may be rapidly degraded in the foreign host, the bacterium may need to be engineered in such a way as to reduce the proteolytic cleavage of the expressed product.

In the case of viral subunit vaccines, prokaryotic expression systems are generally of limited use due to the fact that prokaryotic cells do not post-translationally modify viral proteins correctly. Thus, although the viral proteins or glycoproteins can be produced to high levels in prokaryotic systems and induce very significant immune responses, these immune responses generally do not result in the induction of protective neutralizing antibody. As a result, it is important to express these viral glycoproteins in eukaryotic expression systems. These include yeast, mammalian cells, plants and insect cells [8–11]. All of these systems have demonstrated utility for production of subunit vaccines and, indeed, a number of commercial vaccines are presently being manufactured in yeast [10]. Since each of these systems glycosylate proteins slightly differently, one must consider the economics of each individual system and whether it produces the desired protein modifications. If glycosylation is critical for immunity, mammalian cells may be the best production system. With this in mind, our laboratory developed a non-destructive mammalian expression system to produce secreted glycoproteins. The model virus that we used is bovine herpesvirus. The glycoprotein gene is introduced into Maden Darby bovine kidney cells (MDBK) under the control of the bovine HSP70 promoter [12]. Due to the strength of this promotor, MDBK cells can be incubated at 43°C for 6 h and still maintain viability. Based on this observation, we constructed a MDBK transfected cell line expressing a bovine herpesvirus glycoprotein D (gD) gene without the transmembrane anchor. By removing the transmembrane anchor, the gD is secreted into the extracellular fluid. Secretion of the glycoprotein is critical since the glycoprotein is toxic. Since high intracellular concentrations of gD kill the transfected cells, by secretion, the intracellular concentrations never reach toxic levels. Another advantage of this expression system is that master seed cell lines can be multiplied either in cell factories or fermenters. Once the cells are confluent, they can be maintained in minimal essential media without fetal bovine serum. This dramatically reduces the cost of production and reduces the chances of introduction of extraneous viruses present in bovine serum [13]. Once the cells are confluent, they are incubated at 43°C for 6 h. This step induces the cells to produce large quantities of glycoprotein gD, which is then secreted into the culture media. Following the 6 h incubation, cells are returned to 37°C for 18 h. The cells then secrete the

newly synthesized protein and simultaneously regenerate their vitality. The next day, the culture media containing the secreted glycoprotein gD is removed and the cycle is repeated. We have repeated this cycle over 30 days with limited reduction in viability of the cells and ability to secrete glycoprotein gD. This system has a major advantage over destructive expression systems. With destructive systems, once the culture reaches its maximum number, the cells are destroyed and the antigen is extracted. In contrast, this non-destructive system allows harvesting the vaccine over an extended period of time. A further advantage is the ability to produce the vaccine in the absence of fetal bovine serum. This makes the culture media extremely economical and, more importantly, reduces the cost of downstream processing. It is very difficult to remove fetal bovine serum and albumin from a vaccine preparation. Without the need to conduct expensive downstream processing, this vaccine can be formulated directly into adjuvants and injected into animals. Using this vaccine formulation, we have identified that as little as 10 μg of purified glycoprotein gD is sufficient to induce protection in cattle. Interestingly, the level of serum neutralizing antibody induced by 10 μg of glycoprotein gD was 10 times higher than that induced in animals that recovered from a virulent challenge [14]. A possible reason for this is that bovine herpesvirus contains a number of immuno suppressive proteins which limits the immune response in vivo. Furthermore, these immunosuppressive proteins interfere with other vaccines administered simultaneously, thereby limiting their use in multicomponent vaccines [4]. Thus, another advantage of using subunit vaccines is that by selecting the correct antigen which induces protective immunity, one can eliminate potential immunosuppressive proteins present in the whole pathogen.

In addition to expressing single proteins in different expression systems, it is possible to develop chimeric genes containing the important epitopes from a number of different pathogens. This concept has been coined as a 'string of beads' vaccine [15]. In addition to combining epitopes from a number of pathogens, it is possible to make chimeric proteins containing immunomodulators such as cytokines (interferon gamma and IL-2) that can further enhance the immune response to the individual subunit vaccine. By combining the chimeric protein containing a cytokine with other adjuvants it is possible to enhance the immune response to subunit vaccines 10–20-fold [16]. This ensures that vaccines can be produced much more economically than using conventional approaches.

Subunit vaccines can also be produced by chemical synthesis of short polypeptides. This has been possible through the combination of chemistry, molecular biology and immunology. In order to develop peptide vaccines it is critical to identify conserved epitopes of the pathogen that are required for infection or replication of the pathogen. The advantages of peptide vaccines include: (1) the product is generally easy to produce in large quantities with very little downstream processing required, (2) the vaccine is defined in chemical terms and is free from nucleic acid contamination, thus eliminating the need for rigorous and expensive safety testing, (3) the product is stable indefinitely at ambient temperatures, eliminating the need for maintaining a cold chain from manufacturing to administration, (4) the stability of the product makes it suitable for application in delayed release vehicles which slowly release booster doses of vaccine. One of the major disadvantages of peptide vaccines is their low level of immunogenicity and their rapid degradation in vivo. Indeed, their rapid degradation in vivo may partially explain their low immunogenicity even when incorporated into strong adjuvants or linked to carriers. Recent advances in chemical synthesis to develop retro-inverso peptides are having a significant impact on peptide stability and enhancing immune responses to these new vaccines. Another disadvantage of peptide vaccines is that organisms can mutate in such a way that immune responses to a single epitope can be ineffective. Thus, it is important to ensure the epitope of choice is critical for the survival of the organism.

4. Marker vaccines

A marker vaccine can be either a subunit or a gene-deleted vaccine and is defined as one which can be used in conjunction with a diagnostic test to differentiate a vaccinated animal from a carrier animal. These vaccines are gaining popularity in veterinary medicine where eradication of specific diseases is of national interest.

The technology for developing marker vaccines have been used most extensively for eradication of herpesviruses of pigs and cattle. The basis for marker vaccines is that animals develop immune responses to the antigens that are present in the vaccine but not to antigens from the pathogen that have been excluded from the vaccine (Fig. 1). For example, a subunit vaccine incorporating gD or a gE gene-deleted vaccine can both be used with the same diagnostic kit which tests for the absence of antibodies to gE in animals. In all cases, naturally infected animals develop antibodies to gE and can be considered latent carriers of the virus. In contrast, vaccinated animals do not develop antibodies to gE even though they develop antibodies to other bovine herpesvirus antigens. Since these vaccines reduce shedding from latently-infected animals, it is possible to immunize a herd for a number of years and

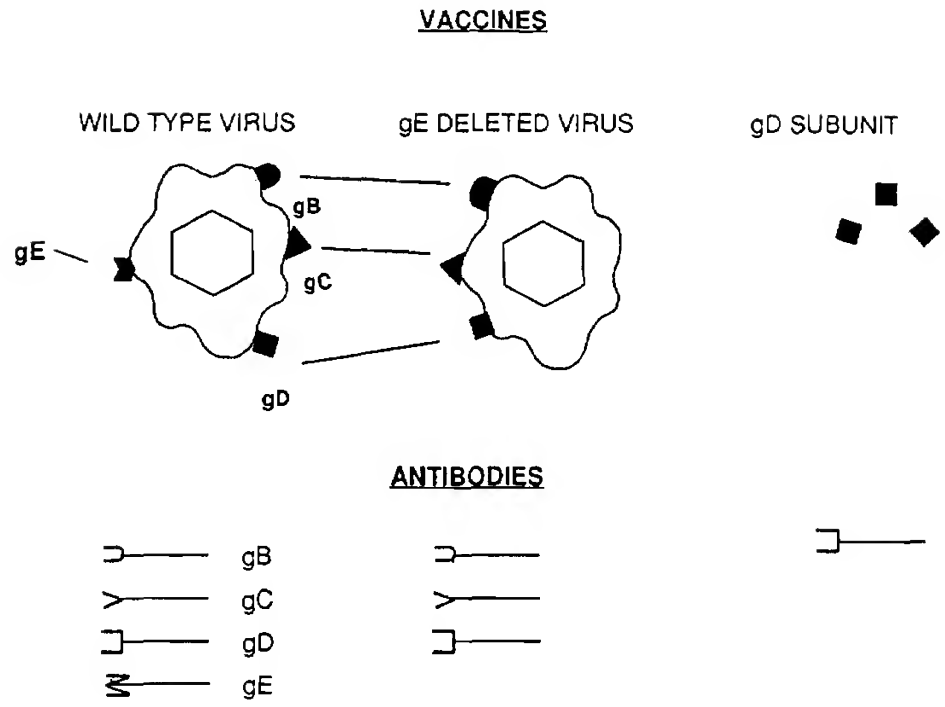

Fig. 1. Marker vaccines. The gene-deleted vaccine and the subunit vaccine do not produce antibodies to gE which can then be used as a marker to differentiate these vaccinated animals from latent carriers.

eventually reduce the shedding to such a level that only the older animals are latently infected [17]. At this time it will be possible to cull the few remaining latent carriers and develop a totally uninfected herd. Countries such as The Netherlands are embarking on a program to eradicate bovine herpesviruses from their borders using this technology. Similar approaches are presently in progress in a number of countries to eradicate pseudorabies. Theoretically, this technology could be used for any viral or bacterial infection provided that the appropriate diagnostics are developed.

These vaccines can also be used following the accidental introduction of an exotic disease into a country. Thus, in addition to quarantine and slaughter, countries can vaccinate animals in the periphery of the actual outbreak and further restrict its spread. Since it is possible to differentiate the vaccinated animals from those which have been exposed to field strains of virus, this approach can assist in eradicating diseases from a country much more rapidly and economically when compared to the present conventional methods of disease eradication. In the case of subunit vaccines, a diagnostic test can be developed against any of the antigens not included in the subunit vaccine. In gene-deleted vaccines the diagnostic test must incorporate the protein encoded for by the deleted gene product. Thus, in both cases a vaccinated animal does not induce antibodies to the marker protein that is absent from the vaccine. One important criteria for choosing a marker protein is that it must be one that induces a rapid and long-lived antibody response in infected animals but not in vaccinated animals even after repeated immunization.

5. Live vaccines

Conventional live viral and bacterial vaccines are produced by selecting avirulent mutants which, although they can cause infection, have a reduced ability to cause disease. These mutations are either induced by treatment of the organism with mutagens, chemicals, heat or passage in vivo or in vitro. Following these mutational events, avirulent mutants are selected and tested in vivo. In most cases, multiple mutations can be induced by this approach and it is often unknown what constellation of genes are altered to achieve attenuation. Using this approach, it is difficult to modulate the degree of attenuation and if the attenuation happens to be due to a single-point mutation, there is a high probability of reversion to virulence following introduction of the vaccine into animals. A well-known example of such a reversion occurs with polio virus where reversion of oral polio vaccines result following administration of the vaccine [18]. This reversion can result in disease in the vaccinee, especially if there is some underlying immunosuppressive condition in the vaccinee. Secondly, the vaccinee sheds virulent virus into the environment which can infect non-vaccinated contacts.

Fortunately, it is now possible to identify specific virulent genes from a wide variety of different pathogens and to directly introduce multiple mutations into these genes or to even delete the entire gene in question. If the virulence gene is the non-essential gene for replication, the gene-deleted pathogen can be used as a vaccine without the fear of causing disease. This approach has a number of advantages. First, the

degree of attenuation can be controlled by deleting or mutating the appropriate genes or group of genes and, secondly, by deleting an entire gene the chance of reversion to virulence is dramatically reduced. If it is possible to identify two non-essential genes that are involved in virulence and delete both of them, then the chances of reversion to virulence is almost nil. These new genetically engineered vaccines should be much safer than the conventional produced live vaccines. Since animals immunized with these gene-deleted vaccines will not produce antibody against the proteins produced by the deleted gene, these vaccines can then be used in conjunction with diagnostic tests to differentiate between animals that are vaccinated and those that are potential carriers of the disease (Fig. 1). The concept of marker vaccines has been described in Section 4.

In addition to deleting non-essential genes, it is possible to even delete an essential gene. These vaccines can be grown in vitro in cell cultures which constitutively express the required gene. For example, if gene X is required for replication of a virus, one can transfect cells in culture with the gene encoding protein X (Fig. 2). By deleting essential gene X from the virus, the virus can still replicate in the transfected cell line, since the cells are providing the essential function. This allows in vitro production of the vaccine. However, upon introduction of the vaccine into an animal, the virus will initiate infection but will only undergo a single abortive cycle of infection. As a result of the abortive infection, the virus will produce all of the viral proteins, that would be recognized by the host immune response. However, since the virus could never complete its replication cycle, due to the absence of the essential gene in the animal, the vaccine is extremely safe and could never be shed into the environment. Such a defective infectious single cycle (DISC) virus is presently being tested as a potential vaccine to control herpesvirus infections [19,20].

In addition to using the genetically engineered gene deleted vaccines to protect individuals against a specific pathogen in question, these genetically engineered strains can also be used as vectors into which foreign genes coding for protective antigens from other pathogens can be introduced. Presently, a variety of viral and bacterial vectors, carrying multiple genes, are being constructed. One of the most successful vectored vaccines is based on the vaccinia virus carrying the rabies virus glycoprotein gene. Since vaccinia virus has a broad host range and is extremely thermal stable, the vaccinia rabies recombinants are being incorporated into baits and distributed from airplanes to vaccinate wildlife reservoirs of rabies [21]. As a result of this approach, a number of Western European countries are dramatically reducing the amount of wildlife rabies as well as reducing transmission of rabies from wild animals to domestic livestock and humans. Without the combination of the stability of vaccinia virus and its ability to express high levels of rabies virus glycoproteins, it would not be possible to immunize individual wildlife species and control rabies virus. The stability and ease of growing vaccinia virus makes it an ideal candidate for controlling a number of diseases [22]. Since vaccinia virus as well as a number of other bacterial pathogens have the capacity to accept a large number of different genes into their genetic material, it should be possible to develop multivalent vaccines to protect individuals against 5–6 different pathogens with a single vaccine. The major advantage of this approach would be that there should be limited interference between the antigens introduced in the multivalent vaccine and, furthermore, these viruses could be delivered to mucosal surfaces to induce both mucosal as well as systemic immunity.

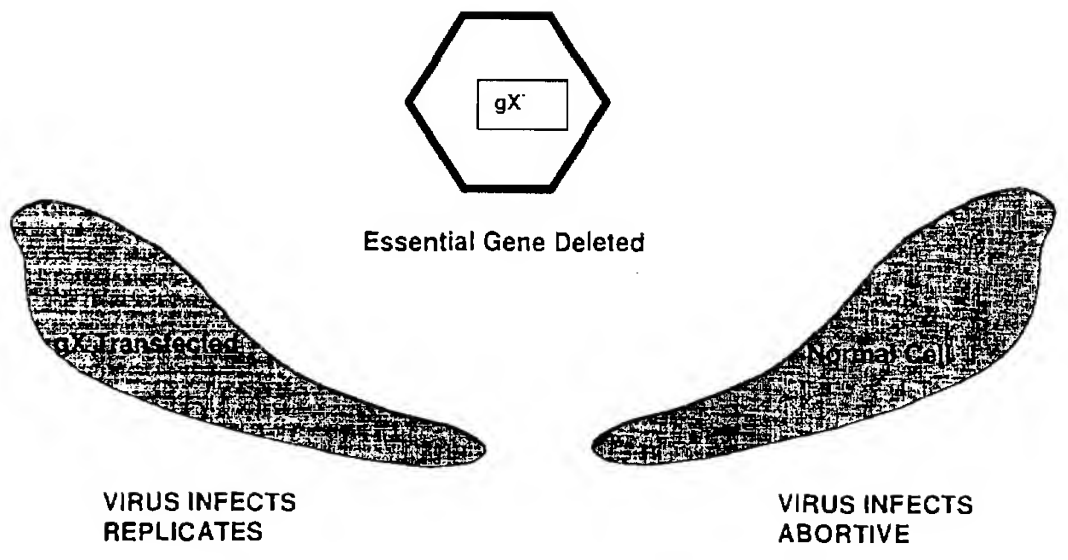

Fig. 2. Defective infectious single cycle (DISC) virus. The virus containing a deleted gene (x) can only replicate in cells constitutively expressing gene X. This allows the production of high titre defective virus. This virus, when administered to an animal, which does not express gene X can infect the cell resulting in an abortive infection. Fortunately, sufficient levels of antigen are expressed to induce an immune response.

Furthermore, since these vaccines would be delivered in a manner which resembles a natural infection, it should also induce a balanced immune response including cell-mediated immunity. Finally, it is possible to introduce genes encoding cytokines into these vectors so as to enhance immune responses to other genes. Since cytokines are central to orchestrating a wide range of immune responses as well as directing specific responses, such an approach will have significant implications on potential vaccination regimes. The major disadvantage of these vaccines is that if individuals are immune to the vector, the immunity response limits the replication of the vector and immunity to the transgene is often reduced. This can be partially alleviated by choosing a vector that the host is not normally exposed to. This is the reason that vaccinia is so effective in immunizing foxes, since foxes are not naturally exposed to vaccinia virus and, therefore, do not have pre-existing immunity to vaccinia.

6. Polynucleotide immunization

The most recent development in vaccinology is immunization with polynucleotides. This technology has also been referred to as genetic immunization or DNA immunization. The basis for this approach to immunization is that cells can take up plasmid DNA and express the genes within the transfected cells. This has been known to occur in vitro for many years and is the basis for in vitro transfection. However, in 1990 Wolff et al. [23] showed that cells in an animal could also take up plasmids and express the genes in vivo. Since genes from any pathogen can be incorporated into a plasmid and if gene expression is regulated by a promotor, it appears logical that expression of the foreign gene in vivo should lead to an immune response to the protein produced by the gene. This was shown to be the case with genes from a wide variety of pathogens including viruses, bacteria and parasites [24–28]. Although it is not possible to quantitate the level of protein produced in vivo, it appears that even very small amounts of protein are sufficient to induce both cellular and humoral immune responses following plasmid introduction into a wide variety of species [29,30]. Indeed, a number of clinical trials are presently in progress in humans and in various animal species in attempts to protect these individuals from a wide variety of pathogens. Thus, the phenomenon of DNA immunization appears to have broad applicability.

The attractiveness of DNA immunization lies in some of the advantages of this technology over other vaccine technologies (Table 2). Indeed, it has been referred to as the 'third generation of vaccinology' because of these advantages. First, since the immune response is induced by a single gene, rather than the whole organism, this approach is safe with regards to ensuring that no infection occurs or spreads from vaccinated animals to contacts. Regulatory agencies have, however, expressed some concern regarding the potential of the plasmid to integrate into host cells and to possibly precipitate aberrant cell division due to insertional mutation, chromosomal alterations or rearrangements, activation of oncogenes, or inactivation of tumor-suppressive genes [31]. Although these concerns are legitimate, the probability of integration appears to be relatively low and can be further reduced by developing plasmids with minimal or no homology to host sequences, thereby reducing the chances of integration. Secondly, regulatory concerns have been raised regarding the possibility of inducing anti-DNA antibodies following DNA immunization. This concern is waning with the discovery that it is very difficult to induce anti-DNA antibodies using the technology employed for DNA immunization. In order to induce DNA antibodies following immunization, one requires administration of the DNA with very strong adjuvants [32,33]. Since DNA immunization does not employ adjuvants, there are also no adverse reactions observed at the injection site as is the case with many vaccines incorporated in adjuvants.

Table 2
Advantages of polynucleotide vaccines

(1) No risk of disease (safety)
(2) Broad range of immune responses (CMI + Ab)
(3) Antigen conformation is normal
(4) No injection site reactions
(5) Duration of immunity is long
(6) Simple, cheap to produce
(7) Multiple vaccines can be administered simultaneously
(8) Neonatal immunization in presence of maternal antibody is possible
(9) Various delivery methods are available

Since prevention of infection and recovery from most diseases requires both cellular and humoral responses, the observation that DNA immunization induces both of these responses is very encouraging. In the case of viral glycoproteins, post-translational modification is important to ensure that the proteins are folded correctly and induce neutralizing immune responses. Since the viral glycoproteins are produced in mammalian cells, the fidelity of these post-translational modifications is great and, more importantly, the antigen is produced endogenously resulting in correct processing of the antigen and presented to the immune system in a manner similar to what occurs following natural virus infection. This leads to induction of cytotoxic T-cells that are responsible for clearing many viral infections. Since some pathogens require a strong Th-1 response, DNA immunization allows this polariz-

ation of the immune response. However, should a Th-2 response be important in providing protection it is also possible to modulate the immune response in such a way that a Th-2 response can be induced. This polarization of the immune response can be influenced by the form of antigen (secreted versus membrane-anchored antigen), route of administration of DNA and by co-administration with genes encoding for cytokines or co-stimulatory molecules [34–37]. Thus, it should be possible to simulate the most appropriate response required for polarization of the immunity as desired. By delivering the plasmids to mucosal surfaces, it is also possible to induce mucosal immune responses [38–42]. Thus, this technology provides an excellent opportunity to tailor the immune response to ensure the most effective protective responses are generated.

One of the important features of an ideal vaccine is duration of immunity. Results presented to-date suggest that DNA immunization, possibly due to the extended expression of proteins by the plasmids, induce immunity of long duration. Whether this will be for the life of the individual is still debated, but it is clear that the duration is longer than that observed following conventional killed or subunit vaccines.

Another major advantage of DNA immunization, especially in livestock, is a potential to induce immune responses in neonates in the presence of maternal antibodies. This provides an opportunity to vaccinate animals at an early age so as to ensure that there is no 'window of opportunity' for the pathogen to establish itself in the neonate. Thus, for the first 2 weeks the animals are protected by passive antibody acquired from the mothers, but as the passive antibody decays, active immunity develops. This also allows immunization of animals at a time when animals are most accessible and easy to handle. Finally, the quantity of DNA required to induce immune responses is relatively low and since production and purification of plasmids are relatively simple, these vaccines should prove to be relatively inexpensive. The economics of production and potential to combine plasmids encoding different genes provide an excellent opportunity to immunize animals against multiple pathogens simultaneously. This should make vaccines for many diseases very economical to produce and deliver.

Although development of delivery systems for DNA vaccines is still in its infancy, a number of novel approaches have proven to be extremely effective and can circumvent the use of needles. More importantly, these systems often reduce the amount of DNA required to induce an immune response. Methods such as intradermal administration of plasmids using a gene gun or other needless injection systems deliver the plasmids to an area of the body rich in antigen-presenting cells [43–45]. The possible disadvantage of this method of delivery in livestock is that delivery cannot occur in areas of the body containing hair. Unfortunately, only a few areas are devoid of hair in livestock. This restricts the ease of immunization by these methods. One of the potential sites of administration of DNA vaccines are mucosal surfaces of the oral and nasal cavities. These sites are accessible to gene gun and needless injection systems. Furthermore. incorporation of the plasmids into liposomes and delivering them to mucosal surfaces has also been reported [40, 41]. Mucosal delivery has another advantage in that delivery at these sites induces mucosal immune responses which are critical for limiting the initial infection by a pathogen at the site of entry.

Although the exact mechanism whereby DNA uptake occurs and, indeed, which cells are important in expressing the antigen for induction of immune response are still controversial, recent advances have begun to demonstrate that both muscle cells as well as professional antigen-presenting cells can take up DNA and initiate the immune response [45]. A thorough review of antigen presentation is summarized in a paper by Dr. Whitton in this volume.

Although immunization has been practiced for over 200 years, the last decade has seen very significant advances in new technologies for the development of new vaccines. These technologies, combined with our understanding of host responses to foreign antigens, has laid the foundation for rapid advances in vaccinology. These successes clearly demonstrate the importance of interdisciplinary research designed to incorporate molecular biology with advances in immunology, immunopathogenesis and chemistry to ensure that better vaccines can be designed and, more importantly, to ensure that the immune response that is important for clearing the pathogen is induced. Based on the progress that has been made in the last decade, it is clear that a new family of vaccines which are safer and more effective will emerge in the next decade.

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